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REMARKS

Claims 1-50 are pending in this application. Claims 36 to 50 are withdrawn from further examination. By this amendment, claims 1, 7, 8, 10-12, 18-22, 26, 28 and 29 have been amended, and claim 5 has been canceled. Support for the amendments can be found, *inter alia*, throughout the specification. Claim 1 is amended to include the recitation in claim 5.

The amendments are made solely to promote prosecution without prejudice or disclaimer of any previously claimed subject matter. With respect to all amendments and cancelled claims, Applicant has not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicant expressly reserves the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Applicant has carefully considered the points raised in the Office Action and believe that the Examiner's concerns have been addressed as described herein, thereby placing this case into condition for allowance.

Restriction Requirement

The Examiner states that the restriction requirement mailed on 7/25/2003 was not an election of species, and the restriction requirement explains why the inventions are independent or distinct and why the search of all inventions would impose a serious burden on the Office.

Applicant disagrees with the Examiner. It is unclear to the Applicant whether it is a restriction requirement for I-LXII or an election of species. In addition, the Examiner did not state why each of I-LXII is able to support separate patents and is independent or distinct. *See* MPEP §803, and §802.01. In this office action, the Examiner examined the full scope of claim 1 as originally filed which includes each mutant SAH hydrolase listed in claim 7. In view of the above,

Applicant respectfully requests that all non-elected mutant SAH hydrolase listed in claim 7 be rejoined.

Objections to the Specification

The Examiner objected to the specification, alleging that the reference to prior applications in the first paragraph of the specification does not contain the current status of all nonprovisional parent applications to which priority is claimed. Applicant notes that the first paragraph of the specification has been amended to reflect the current status of the parent applications.

The Examiner objected to the title, alleging that the title is not descriptive and suggesting to delete the term "compositions" in the title since the elected claims are directed to a method. Applicant notes the title is amended to delete the term "compositions".

The Examiner objected to the specification, alleging that step 1 in Example 4 on pages 44 and 46 does not include the addition of Ado which is required for the formation of SAH from Hcy. Applicant notes that Example 4 describes that the plasma sample was added to TBP and the wild type SAH hydrolase solution, and the wild type SAH hydrolase solution contains 0.25 mM Ado. *See* Specification, page 43, lines 25-26. Thus, Ado was present in the conversion reaction of Hcy to SAH.

Applicant notes that the specification on page 47 is amended to include addition of wild type SAH hydrolase solution. Support for this amendment is found, *inter alia*, in the specification on page 44, lines 20-24.

In view of the above, Applicant respectfully requests that the objections be withdrawn.

Information Disclosure Statement

The Examiner indicated that reference No. 65 was not considered because there is no author's name associated with it. Applicant submits herewith a courtesy copy of the reference No. 65 of December 19, 2002 IDS showing persons responsible for the reference (Exhibit A). The Examiner indicated that reference No. 128 was not considered because its publication date is missing. Applicant submits herewith information from Barnes & Noble showing publication date of reference No. 128 of December 19, 2002 IDS (Exhibit B). Applicant respectfully requests the Examiner consider and make record of reference Nos. 65 and 128 in the present application.

Claim Objections

Claim 7 is objected for allegedly partially drawn to non-elected inventions. The Examiner states that claim 7 will be restricted to the subject matter elected, *i.e.*, method for assaying homocysteine, S-adenosylhomocysteine, or adenosine with a mutant SAH hydrolase comprising the mutation T158Y of SEQ ID NO:1.

As discussed above, Applicant disagrees with the Examiner. Applicant respectfully requests that this objection be withdrawn.

Claim 1 is objected for the recitation of "and said binding affinity and/or said attenuated ..." and for the recitation of "SAH or adenosine, or a combination thereof". Applicant notes that claim 1 is amended as suggested by the Examiner.

Claims 18-22, 28, and 29 are objected for the recitation of "SAH or a derivative or an analogue thereof". Applicant notes that claims 18-22, 28, and 29 are amended as suggested by the Examiner.

Claim 20 is objected for the recitation of "labeled SAH is fluorecin-SAH conjugate or Rocamin-SAH conjugate". Applicant notes that claim 20 is amended as suggested by the Examiner.

Claims 20, 21, and 22 are objected for the recitation of "linker of 1-15 carbon atom length". Applicant notes that claims 20, 21, and 22 are amended as suggested by the Examiner.

Claim 21 is objected for the recitation of "derivative is glucose-6-phosphate dehydrogenase ...". Applicant notes that claim 21 is amended as suggested by the Examiner.

Claim 22 is objected for the recitation of "derivative is bovine albumin-SAH conjugate". Applicant notes that claim 22 is amended as suggested by the Examiner.

Claim 26 is objected for the recitation of "horse radish". Applicant notes that claim 26 is amended as suggested by the Examiner.

In view of the above, Applicant respectfully requests that the objections be withdrawn.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-35 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 2 (claims 3-35 dependent thereon) are rejected for the recitation of "catalytic activity". The Examiner states that since SAH hydrolase can have catalytic activities (e.g., 3'-oxidative activity, 5'-hydrolytic activity, and 3'-reduction activity), one cannot reasonably determine which catalytic activity is being referred to in the claims. The Examiner further states that it will be assumed that the term refers to 3'-oxidative activity, 5'-hydrolytic activity, and 3'-reduction activity for examination purposes.

Applicant respectfully traverses this rejection. Applicant notes that the specification provides that "a mutant SAH hydrolase lacks detectably level of one of its catalytic activities or its overall catalytic activities compared to its wildtype counterpart." *See* Specification, page 12, lines 18-20. The term "catalytic activity" refers to one or more of the catalytic activities of a SAH hydrolase and is sufficiently definite to one skill in the art in view of the specification. Withdrawal of this rejection is respectfully requested.

Claim 2 is rejected for the recitation of "amino acid residue that is directly involved in the SAH hydrolase's catalytic activity". The Examiner alleges that it is unclear which amino acid is being referred to because of use of the term "directly involved", and one cannot determine if the term refers to an amino acid which is essential for the activity recited such that its absence results in lack of activity, or an amino acid whose absence may reduce or increase the desired activity. The Examiner further states both interpretations will be used for examination purposes.

Applicant disagrees with the Examiner. Applicant respectfully notes that the term "directly involved" is clear to one skilled in the art, and the specification also indicates that the term includes both amino acid residues which are essential for the enzymatic activity and amino acid residues whose absence may reduce or increase the desired activity. *See* Specification, page 20, lines 10-24. Accordingly, Applicant respectfully requests that the rejection be withdrawn.

Claims 10 and 11 (claim 12 dependent thereon) are rejected for the recitation of "access adenosine". Applicant notes that claims 10 and 11 have been amended to recite "excess adenosine". Withdrawal of this rejection is respectfully requested.

Claim 12 is rejected for the recitation of "wherein the wild-type SAH hydrolase inhibitor is ...", as allegedly lacking antecedent basis for the term "SAH hydrolase inhibitor". Applicant notes that claim 12 is amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claim 13 (claims 14-15 dependent thereon) is rejected for the recitation of "further comprising the step of removing the reducing agent used to convert oxidized or conjugated ...", as allegedly lacking antecedent basis for the term "reducing agent". Applicant notes that claim 8 is amended to recite that "oxidized or conjugated Hcy in the sample is converted into reduced Hcy by a reducing agent". Withdrawal of this rejection is respectfully requested.

Claim 18 (claims 19-22, 27-29 dependent thereon) is rejected for the recitation of "presence of a labeled SAH, or a derivative or an analogue thereof, thereby the amount of the mutant SAH hydrolase bound to the labeled SAH inversely to the amount of ...". The Examiner

states that it is unclear as to how the amount of the mutant SAH hydrolase bound the labeled SAH can be estimated and it appears from the specification (Example 4, step 6) that the amount to be estimated is the amount of labeled SAH bound to the mutant SAH hydrolase. The Examiner further states that claim 18 does not mention how the labeled SAH derivative or labeled SAH analogue are used in the method. Applicant notes that claim 18 has been amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claims 19-22, 24, 28 and 29 (claims 25-27 dependent thereon) are rejected for the recitation of "fluorescently, enzymatically or proteinaceously labeled". The Examiner states that it is not clear whether the phrase means that the SAH is labeled with a fluorophore, an enzyme, or a protein tag, or means that the SAH is labeled using a fluorescence method, an enzyme, or a protein to attach the label to SAH. Applicant notes that claim 18 has been amended to recite that "wherein the labeled SAH, SAH derivative, or SAH analogue is labeled with a fluorophore, an enzyme, or a protein" as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claim 20 is rejected for the recitation of "wherein the ....labeled SAH is fluorecin-SAH conjugate or Rocamin-SAH conjugate, said fluorecin or Rocamin being linked to said SAH or a derivative or an analogue thereof by a linker ...", as allegedly lacking antecedent basis for "fluorecin" or "Rocamin". Applicant notes that claim 20 has been amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claim 21 is rejected for the recitation of "wherein the ... labeled SAH derivative is ..., said G-6-PDH, alkaline phosphatase, or malate dehydrogenase being linked to said SAH or a derivative or an analogue thereof ...", as allegedly lacking antecedent basis for "G-6-PDH", "alkaline phosphatase", and "malate dehydrogenase". Applicant notes that claim 21 has been amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claim 22 (claim 27 dependent thereon) is rejected for the recitation of "wherein the proteinaceously labeled SAH derivative is bovine albumin-SAH conjugate, said bovine albumin being linked to said SAH or a derivative ...", as allegedly lacking antecedent basis for "bovine

albumin" as it relates to SAH or its analogue. Applicant notes that claim 22 has been amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Claims 28 and 29 are rejected for the recitation of "resulting change of ... is measured for assessing Hcy, SAH or adenosine, as allegedly being unclear which property of Hcy, SAH or adenosine is being assessed. Applicant notes that claims 28 and 29 have been amended as suggested by the Examiner. Withdrawal of this rejection is respectfully requested.

Applicant has attempted to respond to the concerns of the Examiner in order to enhance clarity and to facilitate disposition of the present case. In view of the foregoing, Applicant respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. §112, first paragraph

Written Description

Claims 1-6 and 8-35 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Specifically, the Examiner states that the specification discloses "(1) a method for assaying Hcy, SAH, or adenosine with a mutant SAH hydrolase, wherein said hydrolase comprises the amino acid sequence set forth in SEQ ID NO: 1 and also comprises a substitution at position 302 wherein a phenylalanine has been substituted with a serine (F302S), (2) several mutant hydrolases which comprise the amino acid sequence of SEQ ID NO: 1 and substitutions/deletions at several positions, as indicated in the first column of Table 1, (3) nucleic acids known in the art to encode SAH hydrolases which Applicants assert can be used in obtaining polynucleotides encoding SAH

hydrolases (page 18, first paragraph, page 19, line 3), (4) mutants of the human SAH hydrolase of SEQ ID NO: 1 which have higher binding affinity for SAH (Figure 5), and (5) detection of Hcy concentration by using fluorophore labeled Ado-Cys or Ado-5'ester", but the specification fails to disclose "(a) all other wild-type SAH hydrolases from which mutant SAH hydrolases having the functional characteristics recited in the claims can be made, (b) which mutations in these unknown wild-type SAH hydrolases can be made such that their variants (mutants) display the desired characteristics, (c) the critical structural elements in any polypeptide which are characteristic of any SAH hydrolase, (d) which are the amino acids in any SAH hydrolase that can be substituted, deleted or inserted such that the resulting mutant SAH hydrolases have at least 50 fold increase in binding affinity for Hcy, SAH or adenosine, (e) SAH derivatives or SAH analogs which can be used in the claimed method such that the amount of SAH in a sample can be estimated, or (f) the compounds/directions required for the detection of cholesterol or folic acid after assaying for Hcy, SAH or adenosine in any sample which has been treated such that a mutant SAH hydrolase can be used for the determination of the presence or amounts of Hcy, SAH or adenosine." The Examiner points to three references to support his argument that the art teaches the unpredictability of using structural homology to accurately determine function and even a high degree of structural homology may not result in functional homology. The Examiner contends that one skilled in the art cannot reasonably conclude that Applicant had possession of the claimed invention at the time the instant application was filed in view of the absence of any information correlating structure with the desired functional characteristics, such that one may predict all the members of the genus of compounds recited by the claims, and the disclosure of a few species of compounds which are not representative of all attributes and species within the genus required to practice the claimed invention.

Applicant respectfully traverses this ground for rejection. Applicant notes that claim 1 is amended to recite "a mutant SAH hydrolase derived from a mammalian SAH hydrolase".

The MPEP states, "The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice . . . or by disclosure of relevant identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." MPEP §2162.II.A.3.(a)(ii) [emphasis added] Thus, reduction to practice alone, or disclosure of relevant identifying characteristics alone, is sufficient to satisfy the written description requirement. "Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification." MPEP §2163.II.A.2.

Applicant respectfully notes that the specification teaches a representative number of mutant SAH hydrolases (e.g., mutants shown in Figure 5 and Examples). The specification also provides information correlating structural features with functional characteristics. Crystal structures of a human SAH hydrolase and a SAH hydrolase from rat liver have been determined. *See* Specification, page 19, line 13 to page 20, line 2; Turner et al., *Nat. Struc. Biol.* 5:369-376 (1998) (Reference No. 141 in IDS); Hu et al., *Biochemistry* 38:8323-33 (1999) (Reference No. 218 in IDS). Based on the crystal structures, amino acids that directly and indirectly involved in the SAH hydrolase's catalytic activity and its binding with NAD^+, NADH, Hcy, SAH, and adenosine are determined. *See* Specification, page 20, lines 10 - 24. Since amino acid sequences of SAH hydrolase is highly conserved during evolution (for example, human and rat SAH hydrolase are identical at 97% of amino acid residues and the Dictyostelium and human enzymes are at 75%), amino acid residues involved in binding or catalytic activity of a specific mammalian SAH hydrolase can be determined. *See* Coulter-Karis et al. *Ann. Hum. Genet.* 53:169-75 (1989)

(Reference No. 104 in IDS); Creedon et al., *J. Biol. Chem.* 269:16364-17370 (1994), Fig. 2, and col. 1 on page 16366; and Henderson et al., *Mol. Biochem. Parasitol.* 53: 169-184 (1992), Fig. 3, and col. 1 and col. 2 on page 173. Therefore, the specification provides ample description for correlation between structure and function.

Applicant respectfully notes that the references cited by the Examiner does not support the argument of unpredictability of using information based on known wild type SAH hydrolases and known mutant SAH hydrolase to generate additional mutant SAH hydrolase having the claimed characteristics. Bork states that most prediction schemes and many bioinformatics methods have difficulty exceeding a 70% prediction accuracy in predicting functional and structural features from a sequence with unknown functions. Bork, *Genome Research* 10:398-400 (2000). The conclusion of this reference cannot be applied to generating mutant SAH hydrolase having binding affinity for its substrate but having attenuated catalytic activity. As discussed above, crystal structures of a human SAH hydrolase and a SAH hydrolase from rat liver have been determined. Based on these crystal structures, amino acid residues that are essential to the enzyme catalytic activities and amino acid residues that are directly or indirectly interacting with the substrate analog inhibitor and co-enzyme NAD^+ have been identified. Based on the crystal structures of SAH hydrolase, generally known information of various domains of SAH hydrolases (e.g., catalytic domain and various binding domains including the NAD^+ binding domain), mutant SAH hydrolases can be generated and tested for the claimed characteristics. Applicant has generated many exemplary mutants that can be used for the methods claimed. *See* Specification, page 21, lines 9-25; Fig. 5; and Examples. Thus, this reference cited by the Examiner does not support his argument of unpredictability in the SAH hydrolase art.

In addition, Witkowski et al. and Seffernick et al. cited by the Examiner do not support Examiner's argument of unpredictability of the art. These two references disclose that changing a

few amino acids may change one enzymatic functionality into another enzymatic functionality. These references are not related to SAH hydrolase. Applicant also notes that generating mutant SAH hydrolase having the claimed characteristics only requires attenuating catalytic activity of the enzyme but maintain binding activity of the enzyme, and does not require any change of enzymatic functionality. The Examiner has not provided a reasonable basis that these references support the unpredictability in the art of SAH hydrolase.

"There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed." MPEP §2163.I.A. Applicant respectfully submits that the Examiner has not met the burden to establish lack of written description. In view of the foregoing, Applicant respectfully submits that the written description requirement has been met, and withdrawal of this rejection is respectfully requested.

Enablement

Claims 1-6 and 8-35 were rejected under 35 U.S.C. §112, first paragraph, for allegedly not enabling any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with the claims. Applicant respectfully traverses this rejection.

The Examiner states that "the specification, while being enabling for a method for assaying Hcy, SAH, or adenosine with a mutant SAH hydrolase, wherein said SAH hydrolase comprises the amino acid sequence set forth in SEQ ID NO: 1 and also comprises the substitutions at those positions recited in claim 7 and those positions disclosed in the specification, wherein said mutant SAH hydrolase has attenuated 3'-oxidative activity, 5'-hydrolytic activity, and/or 3' reduction activity, and the same, or higher, binding affinity for Hcy, SAH or adenosine when compared to the polypeptide of SEQ ID NO: 1, wherein the mutant SAH hydrolase can be labeled

and wherein a labeled Ado-Cys or Ado-5'ester can be used, does not reasonably provide enablement for (1) a method for assaying Hcy, SAH, or adenosine using any mutant SAH hydrolase having the functional characteristics recited in the claims, (2) the method of (1) further using any labeled SAH derivative or SAH analog, (3) the method of (1) or (2) further comprising detecting cholesterol and/or folic acid in the sample by any means, or (4) the method of (1) further comprising detecting cholesterol and/or folic acid in a sample by any means, wherein the mutant SAH hydrolase comprises SEQ ID NO: 1 and also comprises the amino acid substitutions recited in claim 7 or in the specification." The Examiner contends that the scope of the claims is not commensurate with the enablement provided "in view of the large number of unknown wild-type SAH hydrolases, unknown mutations in any wild-type SAH hydrolase which would result in the recited functional characteristics, extremely large number of unknown SAH derivatives and SAH analogs, and unknown methods to detect cholesterol and folic acid in a sample which has been treated to interact with a mutant SAH hydrolase for determining presence or amounts of Hcy, SAH or adenosine, required to practice the claimed method." The Examiner further contends that the art cited teaches the unpredictability of isolating proteins of similar function based solely on structural homology and indicates that even high structural homology does not always results in functional homology; and due to the lack of relevant examples, the amount of information provided, the lack of knowledge about the critical structural elements required to display the desired function, and the unpredictability of the prior art in regard to isolation of functional homologs based solely on structure homology, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the full scope of the claimed method. The Examiner concludes that "Applicant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims."

Applicant respectfully traverses this rejection. Applicant notes that claim 1 is amended to recite "a mutant SAH hydrolase derived from a mammalian SAH hydrolase". Applicant respectfully submit that the claimed methods are enabled.

Applicant respectfully notes that the specification provides more than ample guidance for practicing the invention. The specification provides a large number of nucleic acid sequences encoding wild type SAH hydrolase, including several mouse, rat, and human SAH hydrolases. *See* Specification, page 18, line 8 to page 19, line 3. The specification also provides guidelines for making and selecting mutant SAH hydrolase that has binding affinity for Hcy, SAH or adenosine but has attenuated catalytic activity, and wherein said binding affinity and/or said attenuated catalytic activity of said SAH hydrolase is caused by a mutation in said mutant SAH hydrolase's catalytic site, its binding site for NAD^+, NADH, Hcy, SAH, adenosine, or a combination thereof. *See* Specification, page 19, line 6 to page 20, line 7. Methods of generating mutations are well known in the art. As discussed above, crystal structures of a human SAH hydrolase and a SAH hydrolase from rat liver have been determined. Based on these crystal structures, amino acid residues that are essential to the enzyme catalytic activities and amino acid residues that are directly or indirectly interacting with the substrate analog inhibitor and co-enzyme NAD^+ have been identified. *See* Specification, page 20, lines 10-24. Based on the crystal structures of SAH hydrolase, generally known information of various domains and conserved amino acid residues of SAH hydrolases (e.g., catalytic domain and various binding domains including the NAD binding domain), Applicant has generated many exemplary mutants that can be used for the claimed methods claimed. *See* Specification, page 21, lines 9-25; Fig. 5; and Examples. Thus, with the teachings of the specification and knowledge in the art, one skilled in the art would be able to make and selecting mutant SAH hydrolase to practice the invention without undue experimentation.

Applicant respectfully notes that the test for undue experimentation is "not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing *In re Angstadt*, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)). Since, as discussed above, amino acid sequences for SAH hydrolases are highly conserved (e.g., even a leshmanial SAH hydrolase is 70-73% identical to rat and human SAH hydrolases as shown in Henderson et al.), it is routine experimentation for one skilled in the art to identify and clone additional mammalian SAH hydrolase and generating mutant SAH hydrolases. In addition, testing of enzymatic activity and binding activity of a SAH hydrolase are well known in the art, and the specification provides ample teachings and guidance for testing these activities. Since it is routine experimentation to generate additional mutant SAH hydrolase having the claimed characteristics for practicing the methods of the invention, the claimed invention is enabled.

For a *prima facie* case of non-enablement, the burden is on the Office to demonstrate that there is a reasonable basis to question the presumptively sufficient disclosure made by applicant. See, e.g., *In re Wright*, 27 USPQ2d 1510 (Fed. Cir. 1993); MPEP § 2164.04. In other words, the specification must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein. *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971). Furthermore, it is incumbent upon the Examiner to explain why one skilled in the art would doubt the truth or accuracy of any statement in a supporting disclosure and to back up these assertions with acceptable and specific evidence. Id. at 370. Absent evidence to the contrary, the specification must be assumed to be enabling. As discussed above, the three references cited by the Examiner does not support the unpredictability of the art for SAH hydrolase.

Applicant respectfully points out that it is a well-established principle of patent law that "patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art." *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991). In *In re Angstadt*, the Court of Customs and Patent Appeals considered the issue of whether section 112 requires disclosure of a test with every species covered by a claim and concluded that requirement of such a complete disclosure would necessitate a patent application with thousands of examples and "would force an inventor seeking adequate patent protection to carry out a prohibitive number of actual experiments." *In re Angstadt*, 537 F.2d 498, 502 (CCPA 1976). The court concluded that such a requirement would be against public policy because it would have the effect of "depriving inventors of claims which adequately protect them and [would limit] them to claims which practically invite appropriation of the invention while avoiding infringement[, which would] inevitably [have] the effect of suppressing disclosure." *Id.* at 504.

Applicant respectfully notes that the specification does not have to teach what is well known in the art. *See* MPEP 2164.01. Since SAH derivatives and analogs, and methods to detect cholesterol and folic acid are known in the art, Applicant is not required to disclose every known SAH derivatives and analogs, or every known method for detecting cholesterol and folic acid.

In sum, Applicant submits that the pending claims fall within the subject matter that is enabled and described by the specification. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejections Under Nonstatutory Double Patenting

Claims 1-3, 5-6, 8-9, 18-19, 23-24 and 30-34 were rejected under the judicially created doctrine of nonstatutory double patenting as allegedly being unpatentable over claims 1-3, 6-14 and 16 of U.S. Patent No. 6,376, 210.

Applicant will address this issue when otherwise allowable subject matter for this application has been identified.

Applicant notes that claim 7 is amended to delete mutant SAH hydrolases containing mutation N181D or D190A of SEQ ID NO:1. Applicant also notes that claim 7 is not amended to limit to one mutant as suggested by the Examiner.

CONCLUSION

Applicant believes that all issues raised in the Office Action have been properly addressed in this response. Accordingly, reconsideration and allowance of the pending claims is respectfully requested. If the Examiner feels that a telephone interview would serve to facilitate resolution of any outstanding issues, the Examiner is encouraged to contact Applicant's representative at the telephone number below.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no.466992000221.

Dated: June 10, 2004

Respectfully submitted,

By_____

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IUPAC-IUB Commission on Biochemical Nomenclature Symbols for Amino-Acid Derivatives and Peptides[1] Recommendations (1971)

The revised "Tentative Rules" published by CBN in 1966 (1) were an attempt to achieve a broad systematization of various types of abbreviated notation already in use (*e.g.* Brand and Edsall (1947) *Annu. Rev. Biochem.*, 16, 224; Report of the Committee on Abbreviations of the American Society of Biological Chemists, December 18, 1959; Report of the Committee on Nomenclature of the European Peptide Symposium, Pergamon Press, 1963, pp. 261–269; "Tentative Rules for Abbreviations and Symbols of Chemical Names of Special Interest in Biological Chemistry" (2)). They sought to reconcile the needs of the protein chemist, *i.e.* indication of amino-acid sequences, with those of persons concerned more with the chemical reactions of proteins and the synthesis of polypeptides, *i.e.* the need for conveying more detailed chemical information in abbreviated form.

Recent progress in the field of peptide synthesis and in the chemical modification of proteins has made necessary a revision of these "Tentative Rules." This revision has been aided by the work of an expert group consisting of J. S. Fruton, B. S. Hartley, R. R. Porter, J. Rudinger, R. Schwyzer, and G. T. Young. They are greatly indebted to many colleagues, notably W. H. Stein, for helpful suggestions.

1. GENERAL CONSIDERATIONS

1.1 The symbols chosen are derived from the trivial names or chemical names of the amino acids and of chemicals reacting with amino acids and polypeptides. For the sake of clarity, brevity, and listing in tables, the symbols for amino-acid residues, have been, wherever possible, restricted to three letters, usually the first letters of the trivial names.

1.2 The symbols represent not only the names of the compounds but also their structural formulae.

1.3 The amino-acid symbols by themselves represent the amino acids. The use of the symbols to represent the free amino acids is *not* recommended in textual material, but such use may occasionally be desirable in tables, diagrams, or figures. Residues of amino acids are represented by addition of hyphens in specific positions as indicated in Section 3.

1.4 Heteroatoms of amino-acid residues (*e.g.*. O^3 and S^3 of serine and cysteine, respectively, N^6 of lysine, N^2 of glycine, etc.) do not explicitly appear in the symbol; such features are understood to be encompassed by the abbreviation.

1.5 Amino-acid symbols denote the L configuration unless otherwise indicated by D or DL appearing before the symbol and separated from it by a hyphen. When it is desired to make the number of amino-acid residues appear more clearly, the hyphen between the configurational prefix and the symbol may be omitted (see 6.3.1.1 *et seq.*). (Note: The designation of an amino-acid residue as DL is inappropriate for compounds having another amino-acid residue with an asymmetrical center.)

1.6 Structural formulae of complicated features may be used along with the abbreviated notation wherever necessary for clarity.

1.7 All symbols listed below are to be printed or typed as one capital letter followed by two lower-case letters, *e.g.* Gln, not GLN or gln or GlN or glN, regardless of position in a sentence or structure. However, when used for purposes other than to represent an amino-acid residue (*e.g.* to designate a genetic factor), three lower-case italic letters (*i.e. gln*) should be used.

2. SYMBOLS FOR AMINO ACIDS

2.1 *Common Amino Acids*

Alanine	Ala	Leucine	Leu
Arginine	Arg	Lysine	Lys
Asparagine	Asn[2]	Methionine	Met
Aspartic acid	Asp	Phenylalanine	Phe
Cysteine	Cys	Proline	Pro
Glutamic acid	Glu	Serine	Ser
Glutamine	Gln[2]	Threonine	Thr
Glycine	Gly	Tryptophan	Trp (not Try)
Histidine	His	Tyrosine	Tyr
Isoleucine	Ile	Valine	Val

2.2 *Less-Common Amino Acids.* Symbols for less-common amino acids should be defined in each publication in which they appear. The following principles and notations are recommended.

2.2.1 *Hydroxyamino acids*

		Preferred alternatives
5-Hydroxylysine	5Hyl	Lys(5OH) or Lys 5| OH

[1] Document of the IUPAC-IUB Commission of Biochemical Nomenclature (CBN), approved by CBN in May 1971, and published by permission of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry. Reprints may be obtained from Waldo E. Cohn, Director, NAS-NRC Office of Biochemical Nomenclature, Oak Ridge National Laboratory, Box Y, Oak Ridge, Tennessee, 37830, U. S. A.

Comments on and suggestions for future revisions of these Recommendations may be sent to any member of CBN: O. Hoffmann-Ostenhof (Chairman), W. E. Cohn (Secretary), A. E. Braunstein, B. L. Horecker, P. Karlson, B. Keil, W. Klyne, C. Liébecq, E. C. Webb, W. J. Whelan.

[2] Asparagine and glutamine may also be denoted as Asp(NH_2) or Asp, and Glu(NH_2) or Glu, respectively, if necessary (as when the NH_2 is substituted, or its removal or modification is under discussion). See 4.2.

Glx may be used when the residue denoted could be "glutamic acid or glutamine"; similarly, Asx for "aspartic acid or asparagine."

3-Hydroxyproline	3Hyp	Pro(3OH)	OH 3| or Pro
4-Hydroxyproline	4Hyp	Pro(4OH)	or Pro 4| OH

2.2.2 *allo-Amino Acids*

allo-Isoleucine	*a*Ile		OH 5|
allo-Hydroxylysine	*a*Hyl	*a*Lys(5OH)	or *a*Lys

2.2.3 *"Nor" and "Homo" Amino Acids.* "Nor" (*e.g.* in norvaline) is not used in its accepted sense (denoting a lower homologue) but to change the trivial name of a branched-chain compound into that of a straight-chain compound (compare with "iso", paragraph 2.1). "Nor" should therefore be treated as part of the trivial name without special emphasis. "Homo", used in the sense of a higher homologue, may also be incorporated into the trivial name.

Norvaline	Nva	Homoserine	Hse
Norleucine	Nle	Homocysteine	Hcy

2.2.4 *Higher Unbranched Amino Acids.* The functional prefix "amino" is included in the symbol as the letter "A", diamino as "A_2"[3]. The trivial name of the parent acid is abbreviated to two letters. The word "acid" ("-säure", etc.) is omitted from the symbol as carrying no significant information. Unless otherwise indicated, single groups are in the 2 position, two amino groups in the 2 and terminal positions (monocarboxylic acids) or 2 and 2′ positions (dicarboxylic acids). The location of amino groups in positions other than these is shown by appropriate prefixes.

Examples:

2-Aminobutyric acid	Abu
2-Aminoadipic acid	Aad
2-Aminopimelic acid	Apm
2,4-Diaminobutyric acid	A_2bu[3]
2,2′-Diaminopimelic acid	A_2pm[3]
2,3-Diaminopropionic acid	A_2pr[3]

NH_2
3|
or Ala($3NH_2$) or Ala (see 4.3)

β-Alanine	βAla
Ornithine (2,4-diaminovaleric acid)	Orn
6-Aminohexanoic acid	εAhx[4]
3-Aminoadipic acid	βAad

[3] The symbols for diamino compounds previously (1) utilized the letter "D" for "diamino". However, the overuse of D as the initial letter for many compounds beginning with "di" (and of "T" for "tri" and "tetra"), in addition to the fact that standard chemical symbolism utilizes subscript numerals for multipliers, leads to the proposal that diamino should be represented by A_2. This eliminates the ambiguity attached to "D" and makes more clear the chemical relationship between the diamino and monoamino derivatives. It is in keeping with the increasing use of Me_2SO instead of DMSO and of Me_3Si- in place of TMS-, and with the earlier proposal of H_4 for tetrahydro (4).

[4] Recommended in place of the previous (1) εAcp, in which "cp" for caproic may be confused with capric and caprylic.

2.2.5 *N^2-Alkylated Amino Acids.* N^2-Alkylamino acids ar becoming more and more common (e.g. in the large group depsipeptides). This justifies special symbols.

Examples:

N-Methylglycine (sarcosine) (see 6.2)	MeGly or Sar
N-Methylisoleucine	MeIle
N-Methylvaline, etc.	MeVal, etc.
N-Ethylglycine, etc.	EtGly, etc.

2.3 *Nonamino-acid residues linked to peptides.* For residue of muramic, sialic, neuraminic, etc., acids linked to amino-aci residues, as in bacterial-cell-wall components, the symbols Mu Sia, Neu, etc. (preceded by Ac if *N*-acetylated) are recom mended. The symbols for sugar residues (Glc, Gal, etc.) (2 and nucleosides (Ado, Cyd, etc.) (3) may also be used.

3. AMINO-ACID RESIDUES

The links between residues have frequently been shown b peptide chemists as full points (periods, dots: ·) and by carbo hydrate chemists (generally) as short strokes (dashes, hyphens:-) At times, special symbols have been used (> or →) to sho the direction of what is in all cases an unsymmetrical link (pep tide or glycoside).

For consistency and ease of typing as well as economy i printing, the hyphen, representing the peptide bond, should b the standard connecting symbol (2).

The simple usage by which Gly-Gly-Gly stands for glycyl glycylglycine appears to involve the employment of the sam three letters (Gly) for three different residues or radicals (b) (c), (d) below. However, if the dashes or hyphens are consid ered as part of each symbol, we have four distinct forms, for th free amino acid and the three residues, *viz.*:

(a) Gly = NH_2—CH_2—CO_2H the free amino acid
(b) Gly- = NH_2—CH_2—CO— the left-hand unit
(c) -Gly- = –NH—CH_2—CO– the middle unit
(d) -Gly = –NH—CH_2—CO_2H the right-hand unit

For peptides, a distinction may be made between the *peptid e.g.* Gly-Glu (shown *without* dashes at the ends of the symbols) and the *sequence*, *e.g.* -Gly-Glu- (shown *with* dashes at the end of the symbols).

3.1 *Lack of Hydrogen on the 2-Amino Group.* The 2-amin group is understood to be at the left-hand side of the symbo when hyphens are used, and—in special cases—at the point o the arrow when arrows are used to indicate the direction of th peptide bond (–CO → NH–, –NH ← CO–). (For substitutio for 2-amino hydrogen, see 4.1.)

Examples:

-Gly: –$HNCH_2COOH$

>Gly or ⊥Gly: >NCH_2COOH

-Ala: –HNCHCOOH (with CH_3)

>Ala or ⊥Ala: >NCHCOOH (with CH_3)

3.2 *Lack of Hydroxyl on the 1-Carboxyl Group.* The 1-car boxyl group is understood to be on the right-hand side of th symbol when hyphens are employed and—in such special case as 6.3.1.3—at the tail of the arrow when arrows are used to indi

cate the direction of the peptide bond (–CO → NH–, –NH ← CO–).

Example:

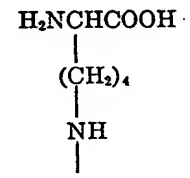
Gly-: H_2NCH_2CO—

It is generally convenient to use the same abbreviated formula for a polypeptide no matter what its state of ionization. To show that a peptide is acting as a cation or anion the amino-terminal and carboxyl-terminal ends of the peptide are amplified with H and OH, respectively (I); these may be modified to show the appropriate state of ionization (II or III).

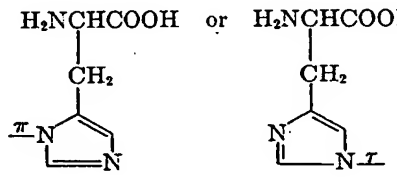
H-Gly-Val-Thr-OH	or	Gly-Val-Thr	(I)
$^+H_2$-Gly-Val-Thr-OH	or	$^+$HGly-Val-Thr	(II)
H-Gly-Val-Thr-O$^-$	or	Gly-Val-ThrO$^-$	(III)

3.3 *Lack of Hydrogen on Amino, Imino, Guanidino, Hydroxyl, and Thiol Functions in the Side Chain* (for substitution in such positions, see 4.2).

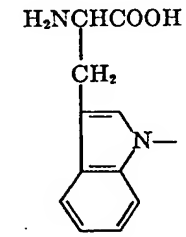
Lys or Lys:



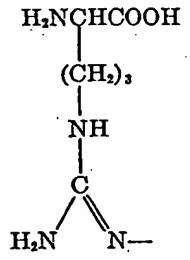
His or His:[5]



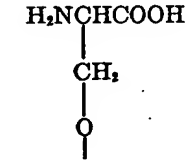
Trp or Trp:



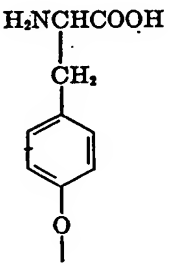
Arg or Arg:



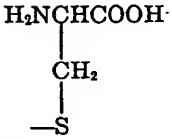
Ser or Ser:



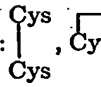
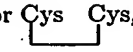
Tyr or Tyr:



Cys or Cys:

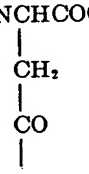


("half-cystine")

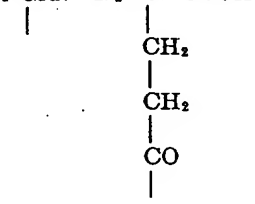
(Cystine would be:  , Cys Cys, or  , *not* Cys–Cys).

3.4 *Lack of Hydroxyl on Carboxyl Groups in the Side Chain.*

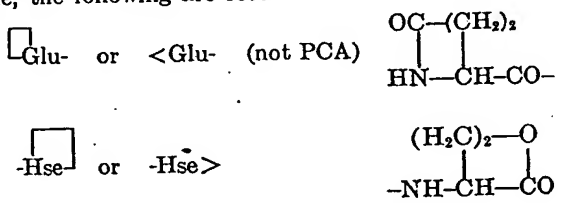
Asp or Asp:



Glu or Glu:



3.5 *Cyclic Derivatives of Amino Acid Residues.* For the special cases of the residues derived from pyrrolid-2-one-5-carboxylic acid (also known as pyroglutamic acid) and from homoserine lactone, the following are recommended:



4. SUBSTITUTED AMINO ACIDS

4.1 *Substitution in the 2-Amino and 1-Carboxyl Groups.* This follows logically from 3.1 and 3.2. The following examples will make the usage clear. (See also 6.2.)

N-Acetylglycine	Ac-Gly
Glycine ethyl ester	Gly-OEt
N^3-Acetyllysine	Ac-Lys
Serine methyl ester	Ser-OMe
O^1-Ethyl *N*-acetylglutamate	Ac-Glu-OEt
Isoglutamine	Glu-NH_2
O^1-Methyl hydrogen aspartate	Asp-OMe
N-Ethyl-*N*-methylglycine	Et-MeGly, $\begin{matrix}\text{Et}\\\text{Me}\end{matrix}$ > Gly, Et Me—Gly

4.2 *Substitution in the Side Chain.* Side-chain substituents may be portrayed above or below the amino-acid symbol (see

3.3 and 3.4), or by placing the symbol for the substituent in parentheses immediately after the amino-acid symbol.

The use of parentheses should be reserved for a *single* symbol denoting a side-chain substituent. Where a more complex substituent is involved, it is recommended that the vertical stroke and the two-line abbreviation be used (5). In general, the one-line abbreviation should be used only when the structure of a substituted peptide is given in textual material.

O^4-Methyl hydrogen aspartate	OMe | Asp or Asp or Asp(OMe) | OMe
O^5-Ethyl hydrogen *N*-acetylglutamate	Ac-Glu(OEt)
N^6-Acetyllysine	Ac | Lys or Lys or Lys(Ac) | Ac
O^3-Acetylserine	Ac | Ser or Ser or Ser(Ac) | Ac
$O^{4'}$-Methyltyrosine	Me | Tyr or Tyr or Tyr(Me) | Me
S-Ethylcysteine	Et | Cys, or Cys or Cys(Et) | Et
S-Sulfocysteine (*S*-Cysteinesulfonic acid)	SO_3H Cys | or | or Cys(SO_3H) Cys SO_3H
Cysteinesulfenic acid	OH Cys | or | or Cys(OH) Cys OH
Cysteinesulfinic acid	O_2H Cys | or | or Cys(O_2H) Cys O_2H
Cysteic acid (3-Sulfoalanine)	O_3H Cys | or | or Cys(O_3H) Cys O_3H
S-Cyanocysteine	CN Cys | or | or Cys(CN) Cys CN
Methionine sulfoxide	O | Met or Met or Met(O)
Methionine sulfone	O_2 | Met or Met or Met(O_2)
O^3-Phosphonoserine (Phosphoserine)	P | Ser or Ser or Ser(P)
N^τ-Methylhistidine[5] (see 3.3) (*tele*methylhistidine)	Me τ| His or His or His(τMe)

similarly for N^π substitution (*pros*methylhistidine)

4.3 *Substitution on Carbon Side Chain.* This may use the same convention as in 4.2, with the addition of locant numerals where necessary, *e.g.*

3-Nitrotyrosine	NO_2 3| Tyr or Tyr or Tyr(3NO_2)
2,3-Diaminopropionic acid (see 2.2.4) (3-aminoalanine)	NH_2 3| Ala or Ala or Ala(3NH_2)
Diiodotyrosine	Tyr(I_2)

5. SYMBOLS FOR SUBSTITUENTS

Groups substituted for hydrogen or for hydroxyl may be indicated either by their structural formulae or by symbols or by combinations of both, *e.g.*

Benzoylglycine (hippuric acid)	–CO-Gly or C_6H_5CO-Gly or [6]Bz-Gly or PhCO-Gly
Glycine methyl ester	Gly-OCH_3 or Gly-OMe
Trifluoroacetylglycine	CF_3CO-Gly

[5] The prolonged and well-entrenched ambiguity in the nomenclature of the *N*-methylhistidines (the chemist's *N*-1 being the biochemist's *N*-3 and *vice versa*) leads to the proposal that a new trivial system for designating these substances is necessary. It is therefore proposed that the imidazole N *near*er the alanine residue be designated *pros* (symbol π) and the one *far*ther *tele* (symbol τ), to give the following names and symbols:
*pros*methylhistidine or N^π-methylhistidine, His(πMe);
*tele*methylhistidine or N^τ-methylhistidine, His(τMe).

[6] Bz- is the symbol generally used for *benzoyl* in organic chemistry. It should not be used for *benzyl* ($C_6H_5CH_2$– or $PhCH_2$–), for which the symbol is Bzl-. However, $PhCH_2$– is unambiguous.

Suggestions for symbols designating substituent (or protecting) groups common in polypeptide and protein chemistry follow.

5.1 *N-Substituents (Protecting Groups) of the Urethane Type.*

Benzyloxycarbonyl-	Z- or Cbz-
p-Nitrobenzyloxycarbonyl-	Z(NO_2)-
p-Bromobenzyloxycarbonyl-	Z(Br)-
p-Methoxybenzyloxycarbonyl-	Z(OMe)-
p-Methoxyphenylazobenzyloxycarbonyl-	Mz-
p-Phenylazobenzyloxycarbonyl-	Pz-
t-Butoxycarbonyl-	Boc- or Bu^tOCO-
Cyclopentyloxycarbonyl-	Poc- or *c*PeOCO-

5.2 *Other N-Substituents.*

Acetyl-	Ac-
Benzoyl- (C_6H_5CO-)	PhCO- or Bz-
Benzyl- ($C_6H_5CH_2$-)	$PhCH_2$- or [6]Bzl
Benzylthiomethyl-	$PhSCH_2$- or Btm-
Carbamoyl-	NH_2CO- (preferred to Cbm)
1-Carboxy-2-nitrophenyl-5-thio-	[7]Nbs-
3-Carboxypropionyl- (HOOC–CH_2–CH_2–CO–)[8]	Suc-
Dansyl- (5-dimethylaminonaphthalene-1-sulfonyl)	Dns-
Dinitrophenyl-	[9]N_2ph- or Dnp
Formyl-	HCO- or CHO-
p-Iodophenylsulfonyl (pipsyl)	Ips
Maleoyl- (–OC–CH=CH–CO–)	–Mal- or Mal<
Maleyl- (HOOC–CH=CH–CO–)	Mal-
Methylthiocarbamoyl-[10]	MeNHCS- or [10]Mtc-
o-Nitrophenylthio-	Nps-
Phenylthiocarbamoyl-[10]	PhNHCS- or [10]Ptc-
Phthaloyl-	–Pht- or Pht<
Phthalyl-	Pht-
Succinyl-[11] (–OC–CH_2–CH_2–CO–)	–Suc- or Suc<
Tetrahydropyranyl-	H_4pyran- (preferred to Thp[9])
Tosyl- (*p*-tolylsulfonyl)	Tos-
Trifluoroacetyl-	[9]CF_3CO-
Trityl- (triphenylmethyl)	Ph_3C- or Trt-

5.3 *Substituents at Carboxyl Group.*

Benzyloxy- (benzyl ester)	–OCH_2Ph or –OBzl
Cyanomethoxy-	–OCH_2CN
Diphenylmethoxy- (benzhydryl ester)	–$OCHPh_2$ or –OBzh
Ethoxy- (ethyl ester)	–OEt
Methoxy- (methyl ester)	–OMe
p-Nitrophenoxy- (*p*-nitrophenyl ester)	–ONp
p-Nitrophenylthio-	–SNp
Phenylthio- (phenylthiolester)	–SPh
1-Piperidino-oxy-	–OPip
8-Quinolyloxy-	–OQu
Succinimido-oxy-	–ONSu
Tertiary butoxy- (*t*-butyl ester)	–OBu^t

[7] See Comment following 5.3.

[8] Not succinyl, although it is the monovalent radical of succinic acid. See succinyl and Footnote 11.

[9] The use of D for "di" and T for "tri" or "tetra" (and DH and TH for "dihydro" and "tetrahydro", respectively) is discouraged. Recognized symbols and subscripts are recommended. See also Footnote 3.

[10] The symbol Pth has been used to denote a phenylthiohydantoin (*e.g.* Pth-Leu). Since this incorrectly implies the substitution of an amino acid by a "phenylthiohydantoyl" group, it is suggested that the abbreviated symbol for such compounds be of the type CS-Leu-NPh or PhNCS-Leu (or Leu>PhNCS in textual material).

[11] Not succinoyl (6).

Comment

Many reagents used in peptide and protein chemistry for the modification (protection) of amino, carboxyl and side-chain groups in amino-acid residues have been designated by a variety of acronymic abbreviations, too numerous to be listed here. Extensive and indiscriminate use of such abbreviations is discouraged, especially where the accepted trivial name of a reagent is short enough, *e.g.* tosyl chloride, bromosuccinimide, trityl chloride, dansyl chloride, etc., or may be formulated in terms of the group transferred, *e.g.* [9]N_2ph-F instead of FDNB for 1-fluoro-2,4-dinitrobenzene, Dns-Cl or dansyl-Cl in place of DNS, [7]Nbs$_2$ in place of DTNB for 5,5′-dithio-bis(2-nitrobenzoic acid) (Ellman's reagent), $(Pr^iO)_2PO$-F, Pr^i_2P-F, iPr_2P-F, or Dip-F[3,9] instead of DFP for diisopropylfluorophosphate. Other commonly-used substances that may be expressed more clearly in terms of symbols are MalNEt (instead of NEM) for *N*-ethylmaleimide, Tos-PheCH_2Cl (instead of TPCK) for L-1-tosylamido-2-phenylethyl chloromethyl ketone, Tos-Arg-OMe (instead of TAME) for tosyl-L-arginine methyl ester, Me_3Si- (instead of TMS-) for trimethylsilyl, CF_3CO- (instead of TFA) for trifluoroacetyl (see 5.2), H_4furan (instead of THF), etc. See also Footnotes 3 and 9.)

Some additional symbolic terms for substituents (and reagents), as examples, are:

2-Aminoethyl-	-$(CH_2)_2NH_2$ (preferred to Aet)
Carbamoylmethyl-	-CH_2CONH_2 (preferred to Cam)
Carboxymethyl-	-CH_2CO_2H (preferred to Cm)
Chloroethylamine	$Cl(CH_2)_2NH_2$
Ethyleneimine	$(CH_2)_2NH$
Chloroacetamide	$ClCH_2CONH_2$
Chloroacetic acid	$ClCH_2CO_2H$
p-Carboxyphenylmercuri-	—HgBzOH
p-Chloromercuribenzoate	*p*Cl-HgBzO⁻
Diazoacetyl-	N_2CHCO—
Hydroxyethyl-	—$(CH_2)_2OH$
Ethylene oxide	$(CH_2)_2O$

6. POLYPEPTIDES

6.1 *Polypeptide Chains* (5). Polypeptides may be dealt with in the same manner as substituted amino acids, *e.g.*

Glycylglycine	Gly-Gly
N-α-Glutamylglycine	Glu-Gly
N-γ-Glutamylglycine	Glu(Gly) or Glu(Gly) or Glu(Gly)
Glutathione	Glu(Cys-Gly) or Glu(Cys-Gly) or Glu(Cys-Gly) or Glu(Cys-Gly)

(Note that Glu would represent the corresponding thiolester with a bond between the γ-carboxyl of glutamic acid and the thiol group of cysteine).

N^2-α-Glutamyllysine Glu-Lys

N^6-α-Glutamyllysine Glu—Lys or Glu(—Lys)—Lys

N^2-γ-Glutamyllysine Glu(—Lys)—Lys or Glu—Lys(—Glu)—Lys

N^6-γ-Glutamyllysine Glu(—Lys)—Lys or Glu—Lys(—Glu)—Lys

The presence of free, substituted or ionized functional groups can be represented (or stressed) as follows:

Glycyllysylglycine H-Gly-Lys-Gly-OH

Its dihydrochloride $^{+}$H-Gly-Lys(+H$_2$)-Gly-OH·2Cl$^-$

Its sodium salt Gly-Lys(Gly-O$^-$ Na$^+$)

Its N^6-formyl derivative Gly-Lys(CHO)-Gly or Gly-Lys(CHO)-Gly

etc.

6.2 *Peptides Substituted at N^2* (see 4.1).

Glycylnitrosoglycine Gly—Gly(NO) or Gly-(NO)Gly

Glycylsarcosine (see 2.2.5) Gly—Gly(Me) or Gly-[Me]Gly or Gly-Sar

N-Glycyl-*N*-acetylglycine Gly—Gly(Ac) or Gly-(Ac)Gly

N,*N*-diglycylglycine Gly(—Gly)—Gly or (Gly)$_2$-Gly

etc.

6.3 *Cyclic Polypeptides.*

6.3.1 *Homodetic cyclic polyptides* (the ring consists of amino-acid residues in peptide linkage only). Three representations are possible:

6.3.1.1 The sequence is formated in the usual manner but placed in parentheses and preceded by (an italic) *cyclo*.

Example: Gramicidin S =

cyclo(-Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro-)

or (see 1.5, sentence 2).

cyclo(-Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu-DPhe-Pro-)

6.3.1.2. The terminal residues may be written on one line, as in 6.3.1.1, but joined by a lengthened bond. Using the same example in the two forms (see 1.5):

└Val-Orn-Leu-D-Phe-Pro-Val-Orn-Leu-D-Phe-Pro┘

or

┌Val-Orn-Leu-DPhe-Pro-Val-Orn-Leu-DPhe-Pro┐

6.3.1.3. The residues are written on more than one line, in which case the CO → NH direction must be indicated by arrows, thus (in the optional manner of 1.5):

┌→ Val → Orn → Leu → DPhe → Pro┐
└Pro ← DPhe ← Leu ← Orn ← Val←┘

6.3.2. *Heterodetic-cyclic polypeptides* (the ring consists of other residues in addition to amino-acid residues in peptide linkage): These follow logically from the formulation of substituted amino acids.

Example:
Oxytocin

Cys-Tyr-Ile-Asn-Gln-Cys-Pro-Leu-Gly-NH$_2$

Cyclic ester of threonylglycylglycylglycine

Thr-Gly-Gly-Gly— or H-Thr-Gly-Gly-Gly—

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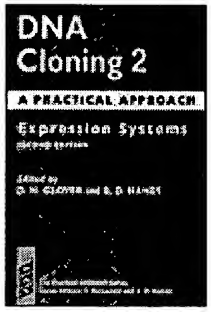
DNA Cloning: Expression Systems, Vol. 2

Hames Glover, David M. Glover (Editor)



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ABOUT THE BOOK

DNA Cloning: Expression Systems, Vol. 2

Annotation

The book contains predominantly black-and-white illustrations, with some color illustra

From the Publisher

This book is the second of a revised set of four Practical Approach volumes on DNA C cover the major experimental techniques in use in modern molecular biology laborator volume, Expression Systems, is written by established researchers in the field. It prov background information and full details of current laboratory procedures for the expres clones in E. coli, lambda-phage, yeast, and baculovirus expression systems, together screening libraries, isolation of proteins on the basis of protein-protein interactions, pu desired protein(s), and the generation of monoclonal and polyclonal antibodies. Each key literature citations to acquaint and inform the reader, together with background inf

date protocols, advice, hints, tips, and troubleshooting comments. Expression System invaluable to PhD students, research scientists, postdoctoral fellows, clinicians, and a involved in molecular biology, genetics, biochemistry, and medical research.

From The Critics

Arshad Rahman

This second edition incorporates contributions from established researchers in the fiel wide range of current laboratory procedures for the expression of cDNA clones in a va expression systems together with the methods for screening libraries, purification of d and the generation of monoclonal and polyclonal antibodies. The purpose is to provide practical guidance in the basic methods needed for controlled production of gene prod largely meets its overall objective. Although intended especially to guide newcomers i involving expression of cloned genes, this book will also prove useful for experienced contributors are active research workers who have important contributions to their res and so have first-hand knowledge of the topics they address. This nicely produced bo collection of established protocols in a step by step, ^^cookbook^^ format. Each chapt brief introduction giving an overview of the methods, followed by a complete list of equ reagents required and an up-to-date protocol. Each chapter concludes with useful hin troubleshooting comments together with a concise list of references for additional info an extremely useful book for anyone interested in learning and mastering the techniqu used for controlled production of gene products. One problem is that the book is boun conventional fashion, as compared to other manuals that come as a spiral notebook. C facilitate its use on a laboratory bench, its natural habitat. Nevertheless, the book help acomprehensive manual designed especially for the novice.

Doody Review Services

Reviewer: Arshad Rahman, PhD (Rush Medical College of Rush University)
Description: This second edition incorporates contributions from established research covers a wide range of current laboratory procedures for the expression of cDNA clon expression systems together with the methods for screening libraries, purification of d and the generation of monoclonal and polyclonal antibodies.
Purpose: The purpose is to provide theoretical and practical guidance in the basic me controlled production of gene products. The book largely meets its overall objective.
Audience: Although intended especially to guide newcomers into studies involving exp cloned genes, this book will also prove useful for experienced researchers. The contri research workers who have important contributions to their respective areas, and so h knowledge of the topics they address.
Features: This nicely produced book is an excellent collection of established protocols step, ^^cookbook^^ format. Each chapter begins with a brief introduction giving an ove methods, followed by a complete list of equipment and reagents required and an up-to Each chapter concludes with useful hints, tips, and troubleshooting comments togethe list of references for additional information.
Assessment: This is an extremely useful book for anyone interested in learning and m techniques currently used for controlled production of gene products. One problem is bound in a conventionalfashion, as compared to other manuals that come as a spiral n binding will facilitate its use on a laboratory bench, its natural habitat. Nevertheless, th the need for a comprehensive manual designed especially for the novice.

Rating

3 Stars from Doody

Accreditation

Glover, D. M. (Univ of Dundee); Hames, B. D. (Univ of Leeds)

The contributors represent the specialties of molecular biology, cell biology, and bioch are from hospitals, academic medical centers, and laboratories in the U.S. and the U.K prominently represented include Massachusetts General Hospital, Univ of Wisconsin Wellcome Research Laboratories, and Univ of Dundee.

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